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PROGRESS REPORT

INCIDENCE OF PINE WOOD NEMATODES IN BARK BEETLE ATTACKED TREES

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Pine wilt disease was first reported from Japan in 1913. The disease is now a major threat to Japan's forest industry and 1980 the Japanese government allocated $35 million to control the disease (Kondo, et al. 1982). The disease is probably caused by a phytotoxin produced by the pinewood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer 1934) Nickle 1970 (Bolla, et al. 1982), a metabolite of an associated bacterium (Oku, et al. 1980), or both.

The disease appears to be endemic in the U.S., not epidemic as in Japan (Dropkin, et al. 1981). *Bursaphelenchus xylophilus* was originally described from specimens collected in Louisiana and is probably native to this continent since many North American *Pinus* spp. exhibit some degree of resistance to the disease. However, it is a potential threat, especially in areas planted with exotic pines or native pines planted on off-site conditions (Wingfield, et al. 1980). Of 30 pine species tested by Futai and Furuno (1979), *Pinus elliottii* Engelm. (slash pine) and *P. taeda* L. (loblolly pine) were found to be highly resistant to pine wilt disease. However, resistance varies inversely with the number of nematodes in the inoculum.

In Japan the worm is vectored by the cerambycid, *Monochamus alternatus* Hope, and in the U.S. the major vectors are the long horn beetles: *Monochamus carolinensis* (Olivier), *M. scutellatus* (Say) and *M. titillator* (F) (Linit, et al. 1983, Williams 1980, Wingfield 1983). In primary infestations, the worm is introduced into wounds made by long horn beetles while feeding on succulent bark (Kondo, et al. 1982). Secondary infestations are produced when female beetles oviposit in dead or dying trees (Wingfield 1983). In the latter case the trees may have been killed or stressed by fungal disease agents, insects or physical factors.

This study was undertaken to determine the incidence of *B. xylophilus* in an active southern pine beetle (*Dendroctonus frontalis* Zimm.) infestation and to note any changes in the incidence of the worm through time.

Materials and Methods

A large southern pine beetle infestation located 6.8 km (4.5 mi.) NW of Williana, Louisiana in the Kisatchie National Forest (Saddle Bayou area) was used for this study. An initial survey for the pinewood nematode was made in July, 1983 by sampling 94 loblolly pine trees that had been or were currently under attack by the southern pine beetle. Samples were taken at a height of 1 m using a brace and a 1.9 cm (0.75 in.) auger bit to drill a 3.8 cm (1.5 in.) deep hole into the xylem. Before sampling, the outer bark and phloem-cambium interface were removed with a chisel. Borings were collected in Ziploc® bags and held in an ice-chest until processed. In the laboratory the wood borings were wrapped in Kimwipes® and soaked in distilled water for 18 to 24 hours. Temporary slide mounts were made of any nematodes found in the water. When only immature aphelencoidid worms were present, pieces of xylem and individual worms were transferred to cultures of *Monilinia fructicola* (Wint.) Honey (brown rot of stone fruits) in an attempt to rear the worms to obtain adults.

One hundred and one uninfested trees located just outside of the advancing edge of the bark beetle infestation were sampled in the same manner. A total of 34 southern pine beetle infested trees not previously sampled were climbed between July 27 and Oct. 24 to determine the distribution of *B. xylophilus* along the bole. The bole was divided into five sampling elevations (sections) based on the total bole length infested with *D. frontalis* using a modified standardizing method (Coulson, et al. 1975, Stephen and Taha 1976). Xylem samples were taken from each height using a brace and bit. All but five of these trees were climbed again 20 days after the initial visit and another set of samples taken from each height. One set of eight trees was sampled on 3 occasions (Sept. 7, 27, Oct. 21).

Results and Discussion

Only 4 of the 94 trees (4.2%) in the initial survey revealed the presence of pinewood nematodes and none of the 101 samples from uninfested trees contained this worm. Of the 34 trees climbed, 38% eventually became infected with *B. xylophilus*. Pinewood nematodes were found in 5.8% of these trees on the first sampling date and in 24.1% on the second sampling date. *Bursaphelenchus xylophilus* was absent from two sections on one tree and one section on another that had tested positive for worms on the first sampling date. None of the 8 trees sampled 3 times were infected on the first sampling date, 3 tested positive for pinewood nematodes on the second visit and 7 contained *B. xylophilus* on the third sampling date. Bluestain (*Ceratocystis* sp.) was associated with pinewood nematodes in only 18.4% of the positive samples.

The incidence of pinewood nematodes tended to increase as the season progressed (Table 1). This may be the result of increased cerambycid activity in the fall. No differences were noted in the incidence of worms among the 5 sample elevations. In Japan *B. xylophilus* is more numerous in the upper portion of the trunk in trees wilting in September and is more uniformly distributed on the bole in trees wilting in March (Kanehori, et al. 1975).

The incidence of pinewood nematodes may have been higher than that indicated in this study since some fungus cultures did not produce worms and others developed high populations of *Rhabdontolaimus frontali* Massey 1974. *Rhabdontolaimus frontali* reproduced more rapidly than *B. xylophilus* and appeared to suppress the numerical increase of *B. xylophilus*.

The absence of pinewood nematodes in healthy trees surrounding the southern pine beetle infestation, the low density of the worms in beetle killed trees and trees under attack in July, and the low incidence of the worm on the first sampling date in those trees sampled between July and October indicate that the trees were not stressed by pine wilt disease prior to being attacked by bark beetles. Wingfield, et al. (1982) found pinewood nematodes only in trees or portions of trees killed by pathogenic fungi or insects. It has also been suggested that *Ips* beetles found in *B. xylophilus* infested trees in Indiana may have been responsible for killing the trees (Marshall and Favinger (1980(1981)). Nematodes were most likely introduced into these dying and dead trees by ovipositing cerambycids. Transmission of this worm to dead and dying trees is an efficient means of perpetuating the population until vectored to healthy host trees (Wingfield 1983). Secondary infestations such as that at Saddle Bayou may also provide a reservoir in which cerambycids and pinewood nematodes reproduce and from which infected sawyers may migrate, vectoring pine wilt disease to healthy stands or nursery plantations.

The spotty distribution of *B. xylophilus* along the bole and the failure of finding worms from sample elevations previously found to be infected, indicate the need for better sampling procedures and the need for additional studies on the epidemiology of the disease in conifers native to North America.

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Table 1 -- Percent of trees sampled between July 27 and October 24 having pinewood nematodes.

Date	No. trees sampled	Percent infected
July 27-29	10	10.0
Aug. 16-18	15	13.3
Sept. 6-8	18	11.1
Sept. 27-28	14	21.4
Oct. 21 & 24	14	57.1